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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*
The purpose of this study is to identify genetic modifiers of cancer risk in women with BRCA1 and BRCA2 mutations. We are using two complementary strategies: 1) association studies in candidate genes from the immune surveillance and DNA damage response pathways and 2) a genome-wide scan using relative pairs with BRCA1 mutations to identify novel regions containing modifier genes. To date we have assembled a case-control sample set of 448 mutation carriers and a relative pairs set of 534 mutation carriers. We have completed a sequencing survey of a panel of immune surveillance genes and determined the population frequency of the variants we identified. We have examined a number of candidate genes and have data suggesting variants in TNF-α, IL-6, XPD and p53 may have a role in altering cancer risk in these high risk women. This work is important not only in leading to more refined cancer risk estimates for women with BRCA1 and BRCA2 mutations, but also will yield candidates for risk alleles in the general population and generate hypotheses for mechanisms that explain these effects. Once these mechanisms have been elucidated, these points in key pathways become excellent targets for preventative and therapeutic intervention.

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INTRODUCTION

The focus of this research study is the identification of genetic factors that influence cancer risk is women with BRCA1 and BRCA2 mutations. As the initial phase of this project we have collected DNA and information from a large retrospective cohort of women with BRCA1 mutations. These have been assembled into two, overlapping study samples: 1) a case control set where all study samples derive from women with BRCA1 mutations and cases those with breast cancer and controls are those carriers that have not developed breast cancer and 2) a relative pairs set were all samples are matched with at least one family member who also has a BRCA1 mutation. These sets are being used with two distinct methodologies to identify genetic modifiers of BRCA1 penetrance, including a candidate gene approach focused on a panel of genes involved in response to DNA damage and of genes important in modulating immune surveillance and a modified linkage approach to identify novel genes. The initial proposal stated that we would focus only on women with BRCA1 mutations, as it was not clear that we would be able to develop a sample with adequate power to include both genes. However as we have been able to do so, we are expanding our analysis of candidate modifier genes to include BRCA2 without a request for additional funds. The linkage approach remains targeted only at BRCA1 mutation carriers because of the larger number of samples required.

PROGRESS REPORT

We have made excellent progress in the first twelve months of this study. Only Task 1 from the approved Statement of Work includes work for this time period, but all Tasks are listed below for clarity.

Task 1: Screening of all genetic variants in a series of candidate genes (Months 1-18).

a. Collection of DNA samples from all collaborators.

This task has been completed. The sample set from which the case-control set for analysis of candidate genes was constructed includes 656 women who inherited germline *BRCA1/2* mutations. These samples were ascertained in a retrospective fashion AFTER identification of the through families with a history of breast and/or ovarian cancer at Creighton University, the Dana Farber Cancer Institute, The University of Michigan, Fox Chase Cancer Center, The University of Pennsylvania, The University of Utah, or Women's College Hospital (Toronto) between 1978 and 1997. These women were self- or physician-referred because of a strong family history of breast and/or ovarian cancer, and provided written informed consent for research under protocols approved by the institutional review boards at each institution. Of these women, 330 (50.3%) had been diagnosed with breast cancer and 326 (49.7 %) were unaffected by breast cancer.

In order to minimize the potential for biases using a retrospectively ascertained cohort, a nested case-control sample was generated using an incidence density sampling design. Women were included as breast cancer cases if they had developed an invasive breast cancer of any stage or grade. Women were excluded as cases if they had undergone (prophylactic) mastectomy or oophorectomy prior to the date of their breast cancer diagnosis, since they may have been at different breast cancer risk than women without

prior surgery. In addition, women were excluded as cases if they had a diagnosis of ovarian cancer prior to the date of their breast cancer diagnosis, since these women may have undergone treatments (e.g., oophorectomy) that also may have changed their breast cancer risk. Control women were matched to cases on their years of birth (± 5 years), age, and mutation status (*BRCA1* or *BRCA2* mutation). Controls were excluded if they had undergone a mastectomy, oophorectomy, or were diagnosed with breast or ovarian cancer prior to the date of the matched case's breast cancer diagnosis. The resulting case control sample of 448 women consisted of 278 breast cancer cases and 170 matched controls. The mean age of breast cancer diagnosis in cases was 39.7years (range: 22-74 years) and the mean age of controls was 41.1 years (range: 19-71 years).

The collection of the relative pairs also is almost completed. We have ascertained 534 BRCA1 mutation carriers from families were they can be matched with a relative pair. These pairs are not matched on affected status or age, but analyses will adjust for these factors. Thus age will be taken into consideration by assignment of liability class and diagnosis will be considered in the genotype/haplotype frequency analysis. In the frequency analysis, genotypes shared more frequently than expected by chance alone between concordant affected pairs will be considered candidates for risk alleles, while genotypes shared more frequently than expected by chance alone between concordant unaffected pairs will be considered candidates for protective alleles. Similar analyses will be done for discordant pairs, with assignment of putative risk category based on whether the frequency is skewed toward the affected or unaffected member of the pair. The percentage of relative pair types in this sample set is as follows: siblings 19%, mother daughter 12%, second degree 18%, third degree 27%, fourth degree 17%, fifth degree or greater 7%.

Subtasks b-f (PCR amplification of variant fragments and microsatellites (b), separation with automated sequencer (c), checking of automated data (d), data analysis (e) and reanalysis if indicated (f)) must be completed sequentially for each gene undergoing analysis before conclusions can be drawn so they are considered together with incomplete analyses as noted.

Immune surveillance genes

We have completed a comprehensive sequence analysis of a large number of immune surveillance genes for the presence of polymorphisms and the frequency of these variants in a control population set has been completed. These data are provided in Table 1 and are currently being prepared for publication.

We also have begun the analysis of these genes in the case control cohort. We began with tumor necrosis factor alpha (*TNFα*) because it has numerous polymorphisms in its DNA sequence and plays a critical role in the immune response to tumors. We have genotyped 223 female *BRCA1*mutation carriers and observed the *TNFα* G-308A polymorphism in 46 (21.5%) carriers. Further, we observed that the A-allele (G/A or A/A) was seen 1.5 times more frequently among women who were affected with breast cancers than in women who were not affected with breast cancer. Finally, we have preliminary evidence that this polymorphism is associated with an earlier age of breast cancer diagnosis in

affected *BRCA1* mutation carriers, suggesting that *TNFα* modifies breast cancer penetrance in this cohort. This study provides the first evidence that *TNFα* could play a role in breast cancer susceptibility.

We also have suggestive evidence that variants in IL-6 may be associated with altered cancer risk in BRCA1 mutation carriers. However additional samples are being typed to determine whether this is a statistically significant result.

DNA damage response genes

The genetic polymorphisms and population frequencies for this subclass of genes has been made publicly available by Dr. Henry Mohrenweiser at Lawrence Livermore National Laboratory thus significantly enhancing the speed at which we could perform these analyses. We have now completed an analysis of XRCC1, XPD and p53.

XRCC1 and XPD

We performed these analyses by genotyping the 414 BRCA1 mutation carriers in the case-control sample set (208 affected with breast cancer and 206 without a breast cancer diagnosis). The genotypes examined to date include polymorphic sites in XRCC1 (exon 6 Arg194Trp and exon 10 Arg399Gln) involved in base excision repair and XPD (exon 6 C>A, 156Arg, exon 10 Asp312Asn, exon 22 C>T, Asp711, and exon 23 Lys751Gln), which is active in nucleotide excision repair and transcription coupled repair. Three of four studied genotypes in XPD showed statistically significant association with breast cancer risk in our case population. Lys allele at Lys751Gln in exon 23 (age-adjusted OR: 1.89; 95% CI: 1.10-3.22), C allele (C>T, Asp711) in exon 22 (OR: 2.02; CI: 1.11-3.66) and C allele (C>A, 156Arg) in the exon 6 (OR: 3.96; CI: 1.92-816) showed association with increased breast cancer risk in BRCA1 mutation carriers. Odds ratios were adjusted for age at time of diagnosis and birth cohort (born before or after 1930). No association between genotypes and breast cancer risk was observed for any of the polymorphisms in the XRCC1 gene studied here.

p53

Based on the knowledge that germline p53 mutations cause Li-Fraumeni syndrome, associated with several cancer types, of which one is breast cancer, for this initial analysis we were interested in a subset of women in the case control sample set that have multiple primary cancers where one is breast cancer. We included data in this analysis not funded by this DOD grant for a comparison group in order to compare results in women with and without BRCA1 mutations as well as with and without multiple primary cancers. Thus, we studied 88 women with breast cancer and a personal or family history of multiple primary cancers (MPC), including ovarian cancer and 84 women with a personal and family history of breast cancer only (BC). All women had been previously screened for germline *BRCA1* and *BRCA2* mutations; 38 (43%) of MPC women and 10 (12%) of BC women had a mutation in one of these two genes. We determined the frequency of deleterious germline *TP53* mutations, as well as the common R72P polymorphism in *TP53* and investigated the association of this polymorphism with the development of cancers in the entire study set. Because BRCA1 and p53 physically and functionally

interact, we also evaluated the association between R72P and breast cancer penetrance in women with known *BRCA1* or *BRCA2* mutations.

The common R72P polymorphism was seen at a frequency of 30.8% in the entire sample. MPC women were more likely to carry the homozygous R72 allele compared to BC women (p=0.02; OR, 2.2, 95% CI: 1.1, 4.3). We also found that the presence of any 72P allele was associated with an earlier age of breast cancer diagnosis among *BRCA1* mutation carriers (p=0.02) providing evidence for R72P polymorphism as a modifier of *BRCA1* penetrance.
Based on these results, we now plan to extend these results to the entire BRCA1/2 mutation carrier sample set.

The remaining tasks will begin as indicated in the time frame on the approved Statement of Work.

KEY RESEARCH ACCOMPLISHMENTS

- Collection of matched case-control set of 448 BRCA1 and BRCA2 mutation carriers
- Collection of a relative pair sample set containing 534 BRCA1 mutation carriers
- Completion of a comprehensive sequencing survey of immune surveillance genes for polymorphic variants
- Analysis of the population frequency of the immune surveillance genetic polymorphisms
- Description of TNF-α as a candidate risk modifier in our set of BRCA1/2 mutation carriers
- Description of XPD as a candidate risk modifier in our set of BRCA1/2 mutation carriers
- Exclusion of XRCC1 as a risk modifier in our set of BRCA1/2 mutation carriers
- Description of the Arg72Pro variant in p53 as a candidate risk modifier in our set of BRCA1/2 mutation carriers

REPORTABLE OUTCOMES

- A manuscript is under preparation describing the immune surveillance gene polymorphisms. This work was presented in abstract form at the Susan B. Komen meeting in November, 2000.

- A manuscript is under preparation describing the XPD and XRCC1 analyses. This work was presented in abstract form in at AACR in March, 2001 and an updated, expanded analysis will be presented at ASHG in October, 2001.

- A manuscript has been submitted describing the p53 polymorphism effect in women with BRCA1/2 mutations and multiple primary cancers

- Patents and/or licenses: None.

- Degrees obtained: None.

- Repositories, data banks and informatics tools: No new ones have been created – this work is being performed retrospectively.

- Funding applied for on the basis of this work: None.

- Employment/research opportunities: One postdoctoral fellow has completed her training with the analysis of the XRCC1 and XPD analyses and has obtained permanent employment based on this work. She will not be working on this project in her new position.

CONCLUSIONS

We conclude, based on the first year of funding for this project that this approach is feasible and likely to yield important results on genetic modifiers of breast cancer risk. We have already generated evidence that genetic variants in TNF-α, IL-6, p53 and XPD may function in this capacity. This work is important not only in ultimately leading to more refined cancer risk estimates for women with BRCA1 and BRCA2 mutations, but will also yield candidates for risk alleles in the general population as well as generate hypotheses for mechanisms that explain these effects. Once these mechanisms have been elucidated, these points in key pathways become excellent targets for preventative and therapeutic intervention.

REFERENCES

None

APPENDICES

Table 1 – List of immune surveillance gene polymorphisms

Appendix 1

Gene	n	Polymorphism	Genotype frequency among Caucasians			Genotype frequency among African Americans			p
			WT/WT	WT/V	V/V	WT/WT	WT/V	V/V	
TNF α	158	G-238A	67(91)	3(4)	4(5)	76(90)	4(5)	4(5)	0.96
	158	G-308A	64(87)	9(12)	1(1)	73(87)	10(12)	1(1)	0.99
	158	C-850T	58(78)	14(19)	2(3)	78(93)	5(6)	1(1)	0.03
	158	C-856A	65(88)	8(11)	1(1)	66(79)	17(20)	1(1)	0.27
	158	153T->C	74(100)	0(0)	0(0)	81(96)	0(0)	3(4)	0.0001
	158	75delG	74(100)	0(0)	0(0)	83(99)	1(1)	0(0)	1
TNF α R	158	G351A	60(81)	14(19)	0(0)	84(100)	0(0)	0(0)	[1] *<0.0001*
	158	C418T	74	0(0)	0(0)	71(85)	13(15)	1(1)	0.0002
IL-6	158	G-174C	27(40)	31(46)	10(14)	70(84)	14(16)	0(0)	[1] *<0.0001*
IL-2	158	T742G	36(49)	33(45)	5(6)	61(73)	20(24)	3(3)	[1] *0.0085*
	158	C1776A	74(0)	0(0)	0(0)	73(87)	10(12)	1(1)	0.0055
Il-1 β	158	C3953T	1(3)	10(34)	18(63)	0(0)	7(27)	19(73)	0.5
	158	C4336T	44(60)	26(35)	4(5)	66(79)	16(19)	2(2)	*0.0327*
IL-1R	158	T11100C	9(36)	12(48)	4(16)	21(75)	4(14)	3(11)	*0.0123*
	158	T8006C	13(39)	15(45)	5(16)	14(54)	12(46)	0	[1] *0.04*
IL-10	158	C-819T	27(90)	3(10)	0(0)	18(64)	10(36)	0(0)	[1] *0.0275*
IL-1 α	158	C4282T	73(99)	1(1)	0(0)	74(88)	6(7)	4(5)	*0.031*
	158	G-35A	73(99)	1(1)	0(0)	77(87)	7(13)	0	0.068
IL-12	158	*T-1172A	74(100)	0(0)	0(0)	83(99)	1(1)	0(0)	1
	158	*A-1522G	74(100)	0(0)	0(0)	77(92)	7(8)	0(0)	[1] *0.0148*
	158	C-1243T	73(99)	1(1)	0(0)	78(93)	6(7)	0(0)	0.12
	158	*T-589G	59(80)	14(19)	1(1)	67(91)	17(9)	0(0)	0.56
	158	*A-175G	74(100)	0(0)	0(0)	83(99)	1(1)	0(0)	1
	158	*T-1603A	72(97)	2(3)	0(0)	75(89)	9(11)	0(0)	[1] 0.062
CTLA4	152	C-318T	55(76)	17(24)	0(0)	79(99)	1(1)	0(0)	[1] *0.0001*
	145	G49A	32(45)	27(39)	11(16)	24(32)	34(45)	17(23)	0.22
	158	W55X	74(0)	0(0)	0(0)	83(99)	1(1)	0(0)	1

* Novel polymorphisms

[1] Values calcualted by Fisher Exact Test